MEMBRANE TRAFFICKING

One step becomes two

The fusion of viral envelopes with endosomal membranes and the release of nucleocapsids into the cytoplasm of host cells were thought to occur in a single step during viral infection. However, in *Nature Cell Biology*, Gruenberg and colleagues describe how this one step can be two.

Vesicular stomatitis virus (VSV) takes advantage of the endocytic pathway to infect host cells, and it was thought that nucleocapsid release occurs immediately after viral-envelope fusion with endosomal membranes, which is triggered by the acidic pH of these endocytic compartments. However, the authors found that viral fusion and viral infection (nucleocapsid release into the cytosol and subsequent viral protein synthesis) are two distinct steps. Viral fusion was triggered before most virions reached late endocytic compartments — the drug-induced depolymerization of microtubules, which are required for transport from early to late endosomes, had no significant affect on viral fusion. By contrast, viral infection was affected by drug-induced microtubule depolymerization.

VSV infection, but not fusion, was also blocked by endocytosed antibodies against lysobisphosphatidic acid (LBPA), which selectively interfere with late endosome function. Together, these observations indicate that viral fusion occurs in multivesicular bodies (MVBs) — transport intermediates between early and late endosomes — and that viral envelopes fuse with intralumenal vesicles, rather than with the endosomal membrane. Electron-microscopy studies confirmed that fusion released viral nucleocapsids into the intralumenal vesicles of MVBs, where they remained hidden, rather than into the cytosol. Viral infection, on the other hand, requires transport to late endosomes and the subsequent delivery of viral nucleocapsids to the cytosol.

Focusing on the latter step, Gruenberg and co-workers showed that, in addition to LBPA, the delivery of viral nucleocapsids to the cytosol depends on the putative LBPA effector Alix. This delivery also seems to be regulated by phosphatidylinositol-3-phosphate (PtdIns3P) signalling (PtdIns3P is abundant in endosomes) through the PtdIns3P-binding sorting nexin Snx16. They conclude that "...the nucleocapsid is exported into the cytoplasm after the back-fusion of internal vesicles with the limiting

PLANT CELL BIOLOGY

Close encounters

The plant hormone auxin controls many different aspects of plant growth and development. It mediates its downstream effects through changes in gene expression by targeting the Aux/IAA transcriptional repressors for ubiquitin-ligase-mediated degradation. Other aspects of auxin signalling, such as auxin perception, are less well understood. But the groups of Ottoline Leyser and Mark Estelle now report the remarkable finding that the F-box protein TIR1, which is part of the ubiquitin ligase complex SCF^{TIR1}, is an auxin receptor.

The two groups had previously shown that auxin promotes the interaction between Aux/IAA proteins and SCF^{TIR1}. Now, using pull-down assays, they have shown that the immunoprecipitated SCF^{TIR1} complexes retain the ability to interact with tagged Aux/IAA protein or peptide in an auxin-dependent manner — indicating that the auxin receptor must be present in the precipitate.

The Estelle group found that the effect of auxin was temperature independent. This, together with the continuous requirement of auxin for the Aux/IAA–SCF^{TIR1} interaction, argued against the possibility that auxin regulates the interaction by causing a stable enzymatic modification of TIR1 or Aux/IAA. Instead, might auxin modulate this interaction by binding SCF^{TIR1} directly? Both groups carried out further pull-down assays, but in the presence of radioactive auxin. Radioactivity was retained in the presence of wild-type Aux/IAA protein, but not with a mutant that was unable to bind SCF^{TIR1}. Competitive binding studies with auxin and related compounds showed that auxin binding to SCF^{TIR1} was specific and dose dependent. They also confirmed that the auxin receptor must be localized to the SCF^{TIR1} complex.

As TIR1 is the only known auxin-specific subunit of the SCF^{TIR1} complex, the two groups tested whether auxin interacted directly with TIR1 — which it did. Leyser, Estelle and co-workers expressed TIR1 in two non-auxin-responsive systems (*Xenopus laevis* and insect cells), prepared cell extracts and used pull-down assays to show that heterologously expressed TIR1 and Aux/IAA proteins interacted in an auxin-specific and dose-dependent manner. However, a TIR1 mutant that lacked the F-box motif — which is required for binding the SCF protein SKP1 (ASK1 in *Arabidopsis thaliana*) — did not respond to auxin. So, auxin-induced binding of TIR1 to Aux/IAA requires the F-box motif. Whether this means that the deleted sequence directly mediates binding, or that the function of TIR1 as an auxin receptor requires its assembly into an SCF complex (or at least its interaction with SKP1), remains an open question.

The identification of TIR1 as an auxin receptor identifies a new mode of SCF regulation that might apply to some of the many uncharacterized SCFs in plants and animals. How auxin stimulates the interaction between SCF^{TIR1} and Aux/IAA is not clear — it could promote a conformational change in SCF^{TIR1} that favours Aux/IAA binding, or auxin might cooperatively bind both TIR1 and Aux/IAA. The authors also suggest that there are additional auxin receptors, including TIR1-related proteins and possibly an extracellular receptor.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPERS Dharmasiri, N. *et al*. The F-box protein TIR1 is an auxin receptor. *Nature* **435**, 441–445 (2005) | Kepinski, S. & Leyser, O. The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature* **435**, 446–451 (2005)

FURTHER READING

Dharmasiri, N. *et al*. Plant development is regulated by a family of auxin receptor F box proteins. *Dev. Cell* 2 June 2005 (doi:10.1016/S153458070500184X)

membrane of late endosomes, and that this process is controlled by the phospholipids LBPA and PtdIns(3)P and their effectors".

This work has shown that VSV entry into cells requires two steps, not one. And, although it is important to remember that this route is not followed by all viruses, it might have been hijacked by other pathogenic agents such as anthrax toxin. In the future, it will be interesting to elucidate the molecular mechanisms that underlie the back fusion of intralumenal vesicles with the limiting membrane of MVBs, which will provide further insights into this increasingly versatile compartment.

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Le Blanc, I. *et al.* Endosome-to-cytosol transport of viral nucleocapsids. *Nature Cell Biol.* 12 June 2005 (doi:10.1038/ncb1269)

SIGNAL TRANSDUCTION

A change in taste

To unleash their full phosphorylating potential, kinases themselves need to be phosphorylated — frequently by autophosphorylation of a residue in the so-called activation loop. Lochhead *et al.* studied two dual-specificity Tyr-phosphorylation-regulated kinases (DYRKs) in a bid to find out precisely how this happens, and found that the kinases initially phosphorylate themselves on Tyr, but later turn their attention to Ser/Thr substrates. Further details of this intricate change in taste are reported in *Cell*.

The authors focused on two *Drosophila melanogaster* DYRKs — dDYRK2 and minibrain (MNB) — and identified Tyr358 in dDYRK2 and Tyr326 in MNB as the only residues that were phosphorylated when the recombinant proteins were expressed in insect cells. Furthermore, phosphorylation of dDYRK2 on Tyr358 was necessary for it to then show Ser/Thr activity towards an exogenous substrate.

DYRK Tyr phosphorylation is thought to occur by autophosphorylation. If this occurs in *trans* (that is, is intermolecular), kinase-inactive forms of the proteins would still be expected to be tyrosine phosphorylated. However, kinase-inactive dDYRK2 or MNB weren't phosphorylated when expressed alone or with their wild-type counterpart, and they couldn't phosphorylate a peptide containing their active loop. This indicates that DYRK phosphorylation occurs through a *cis* (intramolecular) mechanism.

Initially, Lochhead *et al.* carried out *in vitro* kinase assays by incubating dDYRK2 or MNB with γ-^{32}P-ATP to try to identify the Tyr-phosphorylated residues. Instead, they found that only Ser and Thr residues were autophosphorylated. Was this because the Tyr autophosphorylation sites were saturated? To address this, the kinases were treated beforehand with a Tyr phosphatase — this showed that they were resistant to dephosphorylation, with only a 50% reduction after 60 min (probably indicating that the activation loop Tyr was inaccessible). This coincided with a 50% reduction in Ser/Thr kinase activity, confirming the requirement of activation loop Tyr phosphorylation for Ser/Thr activity.

Notably, the DYRKs that were dephosphorylated could not rephosphorylate on Tyr. So Tyr autophosphorylation seemed to be a 'one-off' event. By studying the translation of dDYRK2 *in vitro*, Lochhead *et al.* found that the levels of protein expression increased alongside the amount of Tyr phosphorylation (both were detected after 20 min). They then saw that, although both wild-type and kinase-inactive dDYRK2 co-purified with ribosomes during the *in vitro* translation process, only the wild-type form autophosphorylated on Tyr; translation of all the canonical kinase subdomains was required for this to occur.

Finally, Lochhead *et al.* carried out drug-sensitivity assays. Purvalanol A and TBB are known inhibitors of DYRKs, but only Purvalanol A inhibited dDYRK2 autophosphorylation during *in vitro* translation. This difference in sensitivity of the two drugs implies that the mature, folded DYRK structure differs from the transitional intermediate emerging from the ribosome. Supporting the concept that autophosphorylation of the DYRK activation loop Tyr occurs once only and is mediated by a transition intermediate, Purvalanol-A-treated dDYRK2 translation products were incapable of autophosphorylating on Tyr.

So intramolecular autophosphorylation of DYRK activation loop Tyr residues occurs as a 'one-off' event during protein folding before the nascent polypeptide disengages from the ribosome, but only its mature counterpart can phosphorylate substrates on Ser/Thr. Proof indeed that tastes change over time. The authors speculate that the existence of a transitional intermediate that autophosphorylates is probably not unique to DYRKs.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Lochhead, P. A. *et al.* Activation loop autophosphorylation is mediated by a novel transitional intermediate form of DYRKs. *Cell* **121**, 925–936 (2005)

WEB SITE

Vaughn Cleghon's laboratory: http://www.beatson.gla.ac.uk/cleghon.htm